Published with Open Access at Journal BiNET
Vol. 09, Issue 01: 343-356

Asian Journal of Crop, Soil Science and Plant Nutrition

Journal Home: https://www.journalbinet.com/ajcsp-journal.html

Influence of biofertilizer, zinc and boron on the growth and yield of black cumin (*Nigella sativa* L.)

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Article received: 03.04.2023; Revised: 16.08.2023; First published online: 15 February, 2024.

ABSTRACT

The goal of the study was to investigate the influence of bio-fertilizer, zinc and boron on the growth and yield of black cumin (Nigella sativa L.) where BARI Kalozira-1 (Nigella sativa L.) was used as planting material in this study. Randomized complete block design (factorial) with three replications consisted of two factors: Factor-A: bio-fertilizer (4 levels): T_0= 0 kg ha^{-1} (control), T_1= 4 kg ha^{-1}, T_2= 8 kg ha^{-1}, T_3= 12 kg ha^{-1}; Factor-B: zinc and boron (4 levels): M_0= 0 kg ha^{-1} (control), M_1= Zn_{4kg} ha^{-1}, M_2= B_{2kg} ha^{-1} and M_3= Zn_{4kg} + B_{2kg} ha^{-1}. Data on different growth, yield and yield-contributing parameters of black cumin were recorded and significant variation was observed from different treatments. The highest BCR (2.66) was obtained from the T_3M_3 (M_3= Zn_{4kg} + B_{2kg} ha^{-1}), whereas the lowest BCR (1.76) was from the T_0M_0 treatment combination. It may be concluded that sowing black cumin by providing 12 kg bio-fertilizer with 4 kg zinc and 2 kg boron along with RDF was recorded to be a more suitable practice for getting a higher amount and quality of seed yield of black cumin.

Key Words: *Bio-fertilizer, Zinc, Boron, Growth, Yield and Black cumin*

Cite Article: Banik, N., Khatun, K., Mostarin, T., Hasan, M. J., Alam, M. M., Reza, M. S., Chowdhury, S. M. A. and Islam, M. M. (2024). Influence of biofertilizer, zinc and boron on the growth and yield of black cumin (*Nigella sativa* L.). Asian Journal of Crop, Soil Science and Plant Nutrition, 09(01), 343-356. **Crossref:** https://doi.org/10.18801/ajcsp.090124.42

I. Introduction

Black cumin (*Nigella sativa* L.) is well known as a spice crop in Bangladesh as well as the rest of the world. It is commonly known as 'Kalozira' in Bengali, belongs to the family Ranunculaceae, the buttercup family, and is cultivated in the winter season. It is believed to have originated in western Asia, where it grows wild and cultivated. It is widely cultivated in Southwest Asia, South Europe, Syria, Egypt, Pakistan, India, Iran, Japan, China and Turkey (Shewaye, 2011). Bangladesh covers 2733.39 acres of land, with a total annual production of 1343.83 tons (BBS, 2022). It is grown well in Faridpur, Sariatpur, Madaripur, Pabna, Sirajganj, Jessore, Kusthtia and Natore districts total coverage 14742 hectares of land (DAE, 2017). Black cumin grows 20 to 60 cm tall, taproot system with delicate flowers and fruits are large and the capsule is composed of 3 to 7 united follicles with numerous seeds used as a spice (Abadi et al., 2015). Ustun et al. (2014) reported that the seed contains 30-35 % of oil, which has several uses in the pharmaceutical and food industries and it is appropriately known as the seed of blessing (habbatul barakah) (Srivastava, 2014). The popularity of the plant was highly enhanced by the ideological belief in the herb as a cure for multiple diseases like anti-tumor, anti-diabetic, cardioprotective, gastroprotective, heap to-protective, anti-asthmatic, nephroprotective, anti-inflammatory, neuroprotective, immune-modulatory, anticonvulsant, anxiolytic, antinociceptive, anti-oxidant, anti-oxytocic, contraceptive, antibacterial, antifungal and anthelmintic activities were immensely appreciated. The major medicinal components are thymo-quinone and nigellone (a dimer of thymoquinone) where. Woo et al. (2012) stated that these were attributed to imparting anti-tumor, anti-inflammatory, and anti-diabetic properties. Black cumin seeds have an aromatic odor and bitter taste and are essential in soup, sausages, cheese, cakes, and candies (Behera et al., 2004). In Bangladesh, black cumin was cultivated on 2733.39 acres of land, and the average yield was 491.63 kg per acre (BBS 2022).

Bio-fertilizers are living microorganisms capable of mobilizing nutrients from non-usable to usable forms through biological processes. Asad et al. (2004) reported that in plants, bio-fertilizers increase the content of growth hormones such as IAA and GA, leading to enhancement in the growth of plants that have the ability of N fixing, phosphate solubilizing and plant growth promoting microorganisms (Mahdi et al., 2010). Bio-fertilizers are cost-effective and inexpensive sources of plant nutrients, which are also useful as bio-control agents since they control many plant pathogens. The importance of Zinc as a micronutrient in crop production has increased in recent years, hence considered to be the most yield-limiting micronutrient. Zn deficiency is one of the most common widespread disorders in plants and soils of different regions of Bangladesh. Zinc essentially is employed in the functional and structural components of several enzymes, such as carbonic anhydrase, alcohol dehydrase, alkaline phosphatase, phospholipase, carboxypeptidase (Coleman, 1991) and RNA polymerase (Romheld and Marschner, 1991). The functional role of Zn includes auxins metabolism, Nitrogen metabolism, influence on the activities of enzymes, and cytochrome c synthesis. Boron is an essential micronutrient required by plants in a minimal quantity (El Wahab and Mohamad, 2008), rapidly becoming deficient in soils (Tahir et al., 2009). It has particular importance in retaining flower and fruit setting of legume crops (Zhang, 2001); it regulates carbohydrate metabolism and keeps a role in seed formation (BARC, 2005). Boron is crucial in cell division and pod and seed formation (Vitosh et al., 1997). Boron influences the absorption of N, P, and K, and its deficiency changes the optimum equilibrium of those three macronutrients.

II. Materials and Methods

Experimental site

The yield of black cumin is generally low because it is less cared and mostly grown under rain-fed conditions without fertilizers. Nutrient depletion regularly due to intensive production is higher and faster than the rates of nutrient exhaustion from the soil (Jain *et al.,* 2021). There is a scope for improving the yield of this crop by using organic manures, inorganic manures and bio-fertilizers (Verma et al., 2017), where inorganic fertilizers played a significant role in meeting the crop's nutrient requirement. Bio-ZN liquid has a single population of Z-solubilizing bacteria (*Bacillus endophyticus*), which helps to significantly reduce phosphorus content in black gram seeds compared to the control (Meena *et al.,* 2021). Though Zn has an antagonistic relation with phosphorous, boron helps fruit setting and development. The experiment was carried out at horticultural farm in Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh, located at 23^0 41' N latitude and 90^0 22' E longitude at 8.6 meters above sea level. The land belongs to the Agroecological zone of Modhupur Tract, AEZ-28. The field was situated in the sub-tropical and characterized by high temperatures, heavy rainfall during the Kharif-1 season (March-June), and scanty rainfall during the Rabi season (October-March) associated with moderately low temperatures. The monthly average temperature, humidity, rainfall and sunshine hours prevailed at the experimental area during the cropping season.

Soil condition

The selected land was medium-high land with adequate irrigation facilities. The physical and chemical properties of soil of the experimental site were silty clay loam in texture having pH 5.47 - 5.63. Organic matter content was very low (0.78). The physical composition, such as sand, silt, and clay content, was 28%, 41%, and 31%, respectively.

Table 01. Physical and chemical properties of the initial soil

Characteristics	Value
Particle size analysis	
% Sand	28
% Silt	41
% Clay	31
Textural class	Silty Clay Loam (ISSS)
pH	5.6
Organic carbon (%)	0.46
Organic matter (%)	0.78
Total N (%)	0.033
Available P (ppm)	20.0
Exchangeable K (meq/100 g soil)	0.11
Available S (ppm)	45.0

Source: Soil Resource Development Institute (SRDI)

Seed and fertilizers

BARI Kalozira-1 was used in the experiment as a planting material and the seed was collected from the Regional Spice Research Centre, BARI, Joydebpur, Gazipur. The recommended organic manures were added to the soil of experimental field along with nitrogen (N), phosphorous (P) and potassium (K) except control plot.

Treatments

Two factors were used in the experiment as mentioned below

Factor A (Four levels of Bio-fertilizer)	Factor B (Four levels of Zinc sulphate and Boric acid)
T_0 = control	M_0 = Control
T_1 = 4 kg ha^{-1}	M_1 = Zn (4 kg ha^{-1})
T_2 = 8 kg ha^{-1}	M_2 = B (2 kg ha^{-1})
T_3 = 12 kg ha^{-1}	M_3 = Zn (4 kg ha^{-1}) + B (2 kg ha^{-1})

Design and layout

The experiment consisted of 16 treatment combinations and was laid out in Randomized Complete Block Design (RCBD) with three replications. The total plot number was 16 x 3 = 48. The unit plot size was 2 m x 1.5 m (3.00 m^2). The distance between block to block was 0.5 m and the distance between plot to plot was 0.5 m.

Land preparation

The experimental land was prepared with the help of a power tiller by three successive ploughing and cross-ploughing followed by laddering one month before planting. The corners of the plots were trimmed with a spade. The codes were broken into friable soil and the surface of the soil was leveled. Weeds and crop residues of previous crops were removed from the field. The final plowing and land preparation were done on 10 November 2020. The experimental area was laid out according to the design of the experiment. The unit plot was leveled before seed sowing.

Fertilizer management

At first plowing, cow dung and kitchen compost were applied at 10 and 5 ton ha^{-1}, respectively. The full amounts of triple super phosphate and murate of potash and half of the urea were applied at final land preparation as a basal dose. The rest half of the urea was applied in two equal splits at 25 and 50 days after seed sowing.

Seed sowing

Before seed sowing, the seeds were soaked in water for 12 hours to enhance germination. Seeds were also treated with Bavistin @ 2 g kg^{-1} of seeds before sowing. Sowing was done on 19 November 2020. Seeds were sown according to treatment in rows at the rate of 3 kg ha^{-1}. After sowing, the seeds were covered with soil and slightly pressed by hand. Seeds were also sowed around the experimental plot area to check the border effect. After 10 days of seedling emergence, the seedlings were thinned to maintain the required spacing treatments.

Intercultural Operation

The unhealthy seedlings were replaced by healthy seedlings taken from border plants and optimum plant population was maintained by thinning 15 days after sowing (DAS). Weeding was done as per needed. Irrigation was applied when the field was too dry and proper drainage facilities were developed to avoid stagnation. The field was supervised occasionally to avoid the infestation of weeds, insects and diseases.

Harvesting and Threshing

Harvesting was carried out by hand, following at 80% maturity. First, the border row plants were harvested manually from all sides of each plot, and subsequent plants of the net plot were harvested, excluding the five earlier randomly selected and tagged plants for recording various observations on yield components of parameters. The harvested plants were dried in the open air for five days and weighed as biological yield data. Threshing was done manually by beating the capsules with a wooden stick on a clean and dried floor. Then, seeds were cleaned and dried in a well-ventilated, shady room for three days. The mean weight of seeds from each treatment was recorded in grams for computation of yield data.

Data collection

Five healthy plants were randomly selected in each plot as per treatment. Plastic coated labels were tagged for identification and recording of Plant height, Number of primary branches and secondary branches plant^{-1}, Days to first flowering, Length and breadth of the capsule, Number of seeds capsule^{-1} and capsules plant^{-1}, 1000 seed weight, seed weight plot^{-1}, yield.

Economic analysis

The production cost was analysed to determine the most economical combination of macronutrient and plant spacing. All input costs included the cost for lease of land and interests on running capital in computing the cost of production. The interest was calculated @ 14% in simple rate. The wholesale market price of black cumin was considered for estimating the cost and return. Analyses were done according to the procedure of Alam et al. (1989). The benefit-cost ratio (BCR) was calculated as follows:

$$\textbf{Benefit cost ratio (BCR)} = \frac{\textbf{Gross return per hectare (Tk.)}}{\textbf{Total cost of production per hectate (TK)}} \tag{1}$$

Data analysis

The data collected on different parameters were statistically analyzed to obtain the level of significance using the Statist 10 computer package program. Analysis of variance was done following two factors in randomized complete block design. The mean differences among the treatments were compared by the Least Significant Difference (LSD) test at a 5% level of significance.

III. Results and Discussion

Plant height

Different levels of bio-fertilizers application had a significant influence on plant height of black cumin at different growth stages of black cumin plant (Table 02). At 95 DAS, the highest plant height (54.19 cm) was obtained from T_3 (12 kg biofertilizer ha^{-1}) treatment. Similarly, the lowest plant height (48.27 cm) was recorded from the T_0 (control) treatment at 95 DAS.

Table 02. Effect of biofertilizer and zinc and boron on plant height

Treatments	Plant height (cm)				Treatments	Plant height (cm)			
	35 DAS	55 DAS	75 DAS	95 DAS		35 DAS	55 DAS	75 DAS	95 DAS
T_0	5.18 d	15.76 d	36.25 d	48.27 d	M_0	5.42 d	16.14 d	37.00 d	48.59 d
T_1	5.98 c	17.72 c	37.83 c	50.05 c	M_1	5.99 c	17.16 c	38.62 c	50.01 c
T_2	6.56 b	18.97 b	40.31 b	52.36 b	M_2	6.47 b	19.30 b	39.75 b	52.52 b
T_3	7.09 a	20.19 a	42.03 a	54.19 a	M_3	6.93 a	20.04 a	41.05 a	53.76 a
LSD(0.05)	0.1386	0.1370	0.3505	0.3900	LSD(0.05)	0.1386	0.1370	0.3505	0.3900
CV%	4.21	4.56	8.25	7.25	CV%	4.21	4.56	8.25	7.25

Note: T_0 = 0 kg ha^{-1} (control), T_1 = 4 kg ha^{-1}, T_2 = 8 kg ha^{-1} and T_3 = 12 kg ha^{-1}, M_0 = 0 kg ha^{-1} (control), M_1= $Zn_{4\ kg}$ ha^{-1}, M_2 = $B_{2\ kg}$ ha^{-1} and M_3 = $Zn_{4\ kg}B_{2\ kg}$ ha^{-1}

Bio-fertilizers increase the supply of minerals through the dissolved nutrient solutions plants could take in through their roots. Positive effects of biofertilizer on improving crop growth were due to an increase in nitrogenase activity by vesicular-arbuscular mycorrhizae and the synthesis of growth-promoting substances by phosphate solubilizing bacteria (De et al., 2011). Biofertilizer as growth regulators are responsible for rapid cell multiplication, resulting in vigorous growth by increasing plant height and branch production (Ghosh et al., 2000). The efficiency of biological bacteria (PSB) is to increase P uptake by plants from the soil solution, resulting in higher plant biomass and plant height (Mehta et al., 2012). Different levels of zinc and boron had significant variations in the plant height of black cumin at different growth stages of black cumin plant. Plant height increased with increased plant zinc and boron. At 95 DAS, the highest plant height (53.76 cm) was achieved from M_3 ($Zn_{4kg}B_{2kg}$ ha^{-1}) treatment. Again, the lowest plant height (48.59 cm) was observed from M_0 (control) treatment. It indicates that micronutrients significantly impact plant height, and applying B and Zn is most effective for higher plant height (Ibrahim, 2019; Pariari et al., 2009). The combined effect of different biofertilizers with zinc and boron on the plant height of black cumin was found statistically significant on different days after sowing (DAS) (Table 03). At 95 DAS, the highest plant height (57.45 cm) was obtained from T_3M_3 (12 kg biofertilizer ha^{-1} with $Zn_{4kg}B_{2kg}$ ha^{-1}) treatment combination, and the lowest plant height (46.86 cm) was observed from T_0M_0 (control) treatment combination which was statistically similar to T_0M_1.

Table 03. Combined effect of bio-fertilizers, zinc and boron on plant height at 35 DAS, 55 DAS, 75 DAS and 95 DAS of black cumin (*Nigella sativa* L.)

Treatments	Plant height (cm)			
	35 DAS	55 DAS	75 DAS	95 DAS
T_0M_0	4.70 h	14.32 l	34.84 j	46.86 k
T_0M_1	4.90 h	14.44 l	36.40 hi	47.37 jk
T_0M_2	5.23 g	16.76 j	36.75 h	49.04 hi
T_0M_3	5.88 f	17.51 h	36.98 gh	49.80 gh
T_1M_0	5.25 g	15.70 k	35.86 i	48.11 j
T_1M_1	5.73 f	17.17 i	37.81 f	49.39 hi
T_1M_2	6.25 e	18.77 f	37.75 f	51.17 e
T_1M_3	6.70 d	19.25 e	39.89 d	51.52 e
T_2M_0	5.78 f	16.80 j	37.59 fg	49 .00 i
T_2M_1	6.32 e	18.02 g	39.15 e	50.88 ef
T_2M_2	6.76 cd	19.83 d	41.39 c	53.2 0 c
T_2M_3	7.38 b	21.22 c	43.09 b	56.28 b
T_3M_0	5.95 f	17.74 h	39.71 de	50.37 fg
T_3M_1	7.01 c	18.99 ef	41.10 c	52.42 d
T_3M_2	7.65 ab	21.85 b	43.11 b	56.56 b
T_3M_3	7.77 a	22.17 a	44.21a	57.45 a
LSD (0.05)	0.2771	0.2740	0.7011	0.7800
CV %	4.21	4.56	8.25	7.25

In a column means having similar letter (s) are statistically similar and those having dissimilar letter (s) differ significantly by LSD at 0.05 levels of probability. Note: T_0 = 0 kg biofertilizer ha^{-1} (control), T_1 = 4 kg biofertilizer ha^{-1}, T_2 = 8 kg biofertilizer ha^{-1} and T_3 = 12 kg biofertilizer ha^{-1}, M_0 = 0 kg ha^{-1} (control), M_1 = $Zn_{4\ kg}$ ha^{-1}, M_2 = $B_{2\ kg}$ ha^{-1} and M_3 = $Zn_{4\ kg}B_{2\ kg}$ ha^{-1}

Number of primary branches plant^{-1}

There was a significant variation due to the effect of biofertilizer in the number of primary branches of plant^{-1} (Table 04). The maximum number of primary branches plant^{-1} (7.25) was observed at T_3 (12 kg biofertilizer ha^{-1}) treatment, while T_0 (control) treatment showed a comparatively lower (4.98) number of primary branch plant^{-1} at 95 DAS (Table 04). Microbes like fungi, bacteria, yeasts, actinomycetes, algae, etc are capable of producing auxins, gibberellins, etc., in appreciable quantity during vermicomposting (Arancon et al., 2004), which affects plant growth appreciably (Arancon et al., 2006). Significant variation was found due to the effect of zinc and boron on several primary branches of plant^{-1}. It was observed that the lowest number of primary branches plant^{-1} (5.00) was recorded from M_0 (control) treatment and maximum (6.97) from M_3 ($Zn_{4kg}B_{2kg}$ ha^{-1}) treatment (Table 04). Zinc has an activator of many enzymes including dehydrogenase, anhydrase, and superoxide mutase. Under zinc application, an improved root system helped the plant to better.

Table 04. Effect of biofertilizer, zinc and boron on number of primary branches and secondary branch at 75 DAS and 95 DAS of black cumin (*Nigella sativa* L.)

Treatments	Primary branches		Secondary branches		Treatments	Primary branches		Secondary branches	
	75 DAS	95 DAS	75 DAS	95 DAS		75 DAS	95 DAS	75 DAS	95 DAS
T_0	4.82 d	4.98 d	7.352 d	7.44 d	M_0	4.77 d	5.00 d	7.39 d	7.44 d
T_1	5.60 c	5.71 c	8.25 c	8.35 c	M_1	5.82 c	6.00 c	8.88 c	8.96 c
T_2	6.46 b	6.63 b	9.85 b	10.19 b	M_2	6.58 b	6.59 b	10.02 b	10.06 b
T_3	7.12 a	7.25 a	11.21 a	11.38 a	M_3	6.83 a	6.97 a	10.77 a	10.90 a
LSD (0.05)	0.2093	0.2518	0.2418	0.2247	LSD (0.05)	0.2093	0.2518	0.2418	0.2247
CV %	5.36	6.45	4.95	7.35	CV %	5.36	6.45	4.95	7.35

Note: T_0 = 0 kg biofertilizer ha^{-1} (control), T_1 = 4 kg biofertilizer ha^{-1}, T_2 = 8 kg biofertilizer ha^{-1} and T_3 = 12 kg biofertilizer ha^{-1}: M_0 = 0 kg ha^{-1} (control), M_1 = Zn_{4kg} ha^{-1}, M_2 = B_{2kg} ha^{-1} and M_3 = $Zn_{4kg}B_{2kg}$ ha^{-1} In a column means having similar letter (s) are statistically similar and those having dissimilar letter (s) differ significantly by LSD at 0.05 levels of probability

Absorption of water and other nutrients (Bhutia et al., 2015). The entire favorable effect was also attributed to the fact that zinc was essential in nitrogen metabolism. The favorable effects of boron might be attributed to its involvement in cell division and cell expansion. The beneficial effect of boron on the growth and bulb yield of onions was also reported by Singh and Tiwari (2016). The number of primary branches of plant^{-1} showed significant variation among the treatments due to the combined application of biofertilizer with zinc and boron (Table 05). Maximum primary branches plant^{-1} (8.37) was found from the T_3M_3 (12 kg biofertilizer ha^{-1} with $Zn_{4kg}B_{2kg}$ ha^{-1}) treatment combination, which was statistically similar (8.05) to T_3M_2 (12 kg biofertilizer ha^{-1} with B_{2kg} ha^{-1}) treatment combination. On the other hand, the Minimum number of primary branches plant^{-1} (4.42) was recorded in T_0M_0 (control) treatment combination, which was statistically similar to T_1M_0 (3 kg ha^{-1} with control) and T_0M_1 (control with Zn_{4kg} ha^{-1}) treatment combination (Table 05).

Table 05. Combined effect of biofertilizer with zinc and boron on number of primary branches and secondary branches at 75 DAS and 95 DAS of black cumin (*Nigella sativa* L.)

Treatments	Primary branches		Secondary branches	
	75 DAS	95 DAS	75 DAS	95 DAS
T_0M_0	4.17 l	4.42 k	6.58 j	6.67 j
T_0M_1	4.62 k	4.85 i-k	7.24 hi	7.34 hi
T_0M_2	5.17 ij	5.08 h-j	7.50 gh	7.58 gh
T_0M_3	5.33 hi	5.58 gh	8.08 ef	8.17 f
T_1M_0	4.50 kl	4.58 jk	6.83 ij	6.97 ij
T_1M_1	5.75 f-h	5.83 fg	8.00 ef	8.33 f
T_1M_2	6.17 ef	6.25 d-f	8.92 d	8.83 e
T_1M_3	6.00 e-g	6.17 ef	9.25 d	9.33 d
T_2M_0	4.83 jk	5.17 hi	7.83 fg	7.97 fg
T_2M_1	6.25 de	6.58 c-e	8.42 e	9.58 d
T_2M_2	7.00 c	7.00 c	10.67 c	10.67 c
T_2M_3	7.75 b	7.75 b	12.50 b	12.60 b
T_3M_0	5.58 g-i	5.83 fg	8.33 e	8.25 f
T_3M_1	6.67 cd	6.75 cd	10.25 c	10.58 c
T_3M_2	7.98 ab	8.05 ab	13.00 a	13.17 a
T_3M_3	8.23 a	8.37 a	13.25 a	13.50 a
LSD (0.05)	0.4186	0.5036	0.4836	0.2247
CV %	5.36	6.45	4.95	7.35

Note: T_0 = 0 kg biofertilizer ha^{-1} (control), T_1 = 4 kg biofertilizer ha^{-1}, T_2 = 8 kg biofertilizer ha^{-1} and T_3 = 12 kg biofertilizer ha^{-1}, M_0 = 0 kg ha^{-1} (control), M_1 = $Zn_{4\,kg}$ ha^{-1}, M_2 = $B_{2\,kg}$ ha^{-1} and M_3 = $Zn_{4\,kg}B_{2\,kg}$ ha^{-1}; In a column means having similar letter (s) are statistically similar and those having dissimilar letter (s) differ significantly by LSD at 0.05 levels of probability.

Number of secondary branches plant^{-1}

The maximum number of secondary branch plant^{-1} (11.38) was observed at T_3 (12 kg biofertilizer ha^{-1}) treatment while controlled treatment showed a comparatively lower (7.44) number of secondary branch plant^{-1} at 95 DAS (Table 04). Significant variation was found due to the effect of zinc and boron on the number of secondary branches of plant^{-1}. It was observed that the lowest number of secondary branches plant^{-1} (7.44) was recorded from M_0 (control) treatment and the highest (10.90) from M_3 ($Zn_{4kg}B_{2kg}$ ha^{-1}) treatment (Table 04). Brown et al. (2002) reported that boron deficiency reduces chlorophyll and soluble protein contents in the leaves, and the resultant loss in photosynthetic enzyme activity obstructs the plant reaction and decreases net photosynthesis as a result of its effects on the growth and yield of the plant. The number of secondary branches of plant^{-1} showed significant variation among the treatments due to the combined effect of biofertilizer with zinc and boron. The highest secondary branches plant^{-1} (13.50) was found from the T_3M_3 (12 kg bio fertilizer ha^{-1} with $Zn_{4kg}B_{2kg}$ ha^{-1}) treatment combination which was statistically identical to T_3M_2 (12 kg bio fertilizer ha^{-1} with B_{2kg} ha^{-1}) treatment combination. On the other hand, the lowest value for secondary branches plant-1 (6.67) was recorded in T_0M_0 (control) treatment combination, which was statistically similar to T_1M_0 (3 kg ha^{-1} with control) treatment combination (Table 05).

Days to first flowering

There was a significant variation due to the effect of bio-fertilizer in the days of the first flowering. Application of 12 kg bio-fertilizer ha^{-1} at T_3 treatment took the earliest days (57.73) to reach 1st flowering stage. The T_0 (control) treatment was found in the maximum flowering (60.48 days) (Figure 01). Yimam et al. (2015) reported that days to flowering decrease with increased nutrient availability. Different zinc and boron doses had a significant effect on days prior to first flowering (Figure 02). Higher zinc and boron doses at M_3 ($Zn_{4kg}B_{2kg}$ ha^{-1}) treatment took a minimum of days (57.29 days) to reach 1st flowering stage. On the other hand, lower zinc and boron doses at M_0 (control) treatment took maximum days (60.87 days) to reach 1st flowering stage. The combined effect of different biofertilizers with zinc and boron was a statistically significant influence with respect to days to first flowering (Table 06). The maximum days (61.58 days) taken to first flowering at T_0M_0 (control) treatment combination. T_3M_3 (12 kg biofertilizer ha^{-1} with $Zn_{4kg}B_{2kg}$ ha^{-1}) treatment combination took minimum days (55.67 days) to first flowering.

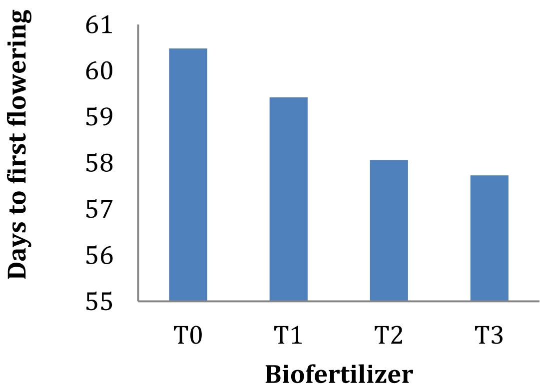

Figure 01. Effect of biofertilizer on days to first flowering of black cumin (*Nigella sativa* L.) (LSD value=0.2111)
Note: T_0= 0 kg biofertilizer ha^{-1} (control), T_1= 4 kg biofertilizer ha^{-1}, T_2= 8 kg biofertilizer ha^{-1} and T_3= 12 kg biofertilizer ha^{-1}

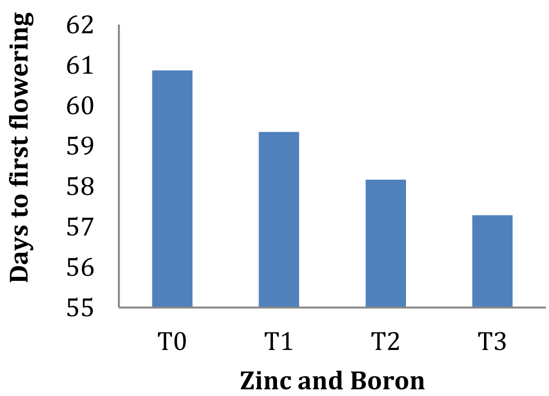

Figure 02. Effect of zinc and boron combination on days to first flowering of black cumin (*Nigella sativa* L.) (LSD value=0.2111)
Note: M_0= 0 kg ha^{-1} (control), M_1= $Zn_{4\ kg}$ ha^{-1}, M_2= $B_{2\ kg}$ ha^{-1} and M_3= $Zn_{4\ kg}B_{2\ kg}$ ha^{-1}

Number of capsules per plant

The number of capsules plant^{-1} was significantly affected by applying different levels of bio-fertilizer (Table 06). The number of capsules plant^{-1} increased with the increased level of bio-fertilizer. The maximum number of capsules plant^{-1} (21.50) was recorded at T_3 (12 kg bio-fertilizer ha^{-1}) treatment. The lowest number of capsules plant^{-1} (16.39) was recorded from T_0 (control) treatment. From the results of the present study number of capsules plant^{-1} increased with the increase in bio-fertilizer doses. Applying bio-fertilizer showed a significant increase in plant height and number of plant^{-1} branches compared to those obtained without bio-fertilizer treatment (control) (Ghosh et al., 2000). Black cumin symbiosis with micro-organisms increased the vegetative growth and the number of lateral branches and capsules per plant. The co-existence relation in plants caused the production of growth-stimulating hormones (Faravani et al., 2012). Significant variations were evident in the case of the number of capsules plant^{-1} with different plant zinc and boron (Table 06). The maximum number of capsules plant^{-1} resulted from M_3 ($Zn_{4kg}B_{2kg}$ ha^{-1}) treatment (21.65) and the lowest (16.28) was obtained from M_1 (control) treatment. The highest fruit set percentage might be due to optimum boron application, as boron plays an important role in maintaining cell integrity, improving respiration, enhancing metabolic activities, and uptake of nutrients. Therefore, plant nutrition with zinc and iron increases pollen carbohydrates storage, prolonging its longevity and thereby increasing pollination and ultimately increasing the number and weight of seeds. The combined effect of different bio-fertilizers and zinc and boron was statistically significant in respect of the number of capsules plant^{-1} (Table 07). The highest number of capsules plant^{-1} (25.00) obtained from the T_3M_3 (12 kg bio-fertilizer ha^{-1} with $Zn_{4kg}B_{2kg}$ ha^{-1}) treatment combination which was statistically similar (24.42) to T_3M_2 (12 kg bio-fertilizer ha^{-1} with B_{2kg} ha^{-1}) treatment combination. The lowest number of capsules plant^{-1} (15.43) was obtained from T_0M_0 (control) treatment combination, which is statistically similar to T_1M_0 (3 kg ha^{-1} with control) treatment combination.

Length of capsule (cm)

There was no significant variation in the capsule length due to biofertilizer application. However, T_3 (12 kg biofertilizer ha^{-1}) treatment had the longest capsule length (1.37cm) and the smallest capsule length (1.11 cm) was obtained from T_0 (control) treatment (Table 07). There was no significant variation in the capsule length due to plant zinc and boron application. The longest capsule length (1.35 cm) was obtained from T_3 (20 cm × 15 cm) treatment and the smallest capsule length (1.14 cm) from T_0 (20 cm × 10 cm) treatment (Table 07).

Table 06. Combined effect of bio-fertilizers and zinc and boron on days to first flowering number of capsule plant^{-1}, length of capsule and breadth of capsule of black cumin (*Nigella sativa* L.)

Treatments	Days to first flowering	Number of Capsule Plant^{-1}	Length of Capsule (cm)	Breadth of Capsule (cm)
T_0M_0	61.58 a	15.43 i	1.06	0.70
T_0M_1	61.15 b	16.15 h	1.08	0.74
T_0M_2	60.17 d	16.92 fg	1.16	0.77
T_0M_3	59.00 e	17.08 efg	1.15	0.78
T_1M_0	60.83 bc	15.83 hi	1.08	0.72
T_1M_1	59.42 e	17.75 e	1.21	0.77
T_1M_2	59.25 e	18.83 d	1.24	0.80
T_1M_3	58.17 f	20.67 c	1.31	0.88
T_2M_0	60.42 cd	16.50 gh	1.17	0.77
T_2M_1	58.50 f	19.08 d	1.28	0.84
T_2M_2	57.00 g	21.33 c	1.33	0.89
T_2M_3	56.33 h	23.83 b	1.46	0.99
T_3M_0	60.67 c	17.33 ef	1.24	0.83
T_3M_1	58.33 f	19.25 d	1.30	0.86
T_3M_2	56.25 h	24.42ab	1.47	1.01
T_3M_3	55.67 i	25.00 a	1.48	1.02
LSD (0.05)	0.4221	0.6739	0.7350 ^{NS}	0.4697 ^{NS}
CV %	4.85	4.25	5.68	7.23

Note: T_0 = 0 kg biofertilizer ha^{-1} (control), T_1 = 4 kg biofertilizer ha^{-1}, T_2 = 8 kg biofertilizer ha^{-1} and T_3 = 12 kg biofertilizer ha^{-1}, M_0 = 0 kg ha^{-1} (control), M_1 = Zn_{4kg} ha^{-1}, M_2 = B_{2kg} ha^{-1} and M_3 = $Zn_{4kg}B_{2kg}$ ha^{-1} In a column means having similar letter (s) are statistically similar and those having dissimilar letter (s) differ significantly by LSD at 0.05 levels of probability.

Amin et al. (2004) reported that applying Zn and B fertilizers, in addition to N and P showed a significant increase in yield and yield contributing attributes. Similar findings were also observed by Alam and Islam (2016), Choudhary et al. (2015) and Rana et al. (2020). The combined effect of different levels of biofertilizer with zinc and boron proved no significant differences in the length of the capsule (Table 07). The longest capsule length (1.48 cm) was obtained from T_3M_3 (12 kg biofertilizer ha^{-1} with $Zn_{4kg}B_{2kg}$ ha^{-1}) treatment combination and the smallest capsule length from T_0M_0 (control) treatment combination (1.06 cm).

Table 07. Effect of bio-fertilizers, zinc and boron on number of capsule plant^{-1}, length of capsule and breadth of capsule of black cumin (*Nigella sativa* L.)

Treatment	Number of Capsule/Plant	Length of Capsule (cm)	Breadth of Capsule (cm)	Treatment	Number of Capsule/Plant	Length of Capsule (cm)	Breadth of Capsule (cm)
T_0	16.39 d	1.11	0.75	M_0	16.28 d	1.14	0.76
T_1	18.27 c	1.21	0.79	M_1	18.06 c	1.22	0.80
T_2	20.19 b	1.31	0.87	M_2	20.37 b	1.30	0.87
T_3	21.50 a	1.37	0.93	M_3	21.65 a	1.35	0.92
LSD (0.05)	0.3369	0.3675^{NS}	0.2349^{NS}	LSD (0.05)	0.3369	0.3675^{NS}	0.2349^{NS}
CV %	4.25	5.68	7.23	CV %	4.25	5.68	7.23

Note: T_0 = 0 kg biofertilizer ha^{-1} (control), T_1 = 4 kg biofertilizer ha^{-1}, T_2 = 8 kg biofertilizer ha^{-1} and T_3 = 12 kg biofertilizer ha^{-1}; M_0 = 0 kg ha^{-1} (control), M_1 = Zn_{4kg} ha^{-1}, M_2 = B_{2kg} ha^{-1} and M_3 = $Zn_{4kg}B_{2kg}$ ha^{-1} In a column means having similar letter (s) are statistically similar and those having dissimilar letter (s) differ significantly by LSD at 0.05 levels of probability

Breadth of capsule (cm)

The longest capsule breadth (0.93 cm) was recorded from T_3 (12 kg bio-fertilizer ha^{-1}) treatment whereas the lowest capsule breadth (0.75 cm) was obtained from T_0 (control) treatment (Table 07). On the other hand, there was no significant variation due to the application of zinc and boron in the breadth of the capsule. The longest capsule breadth (0.92 cm) was obtained from M_3 ($Zn_{4kg}B_{2kg}$ ha^{-1}) treatment and the smallest capsule breadth (0.76 cm) from M_0 (control) treatment. The combined effect of different levels of bio-fertilizer with zinc and boron proved no significant differences in the breadth of the capsule (Table 07). The longest capsule breadth (1.02 cm) was obtained from T_3M_3 (12 kg bio-fertilizer ha^{-1} with $Zn_{4kg}B_{2kg}$ ha^{-1}) treatment combination and the smallest capsule breadth from T_0M_0 (control) treatment combination (0.70 cm). Uwakiem (2022) also observed similar findings.

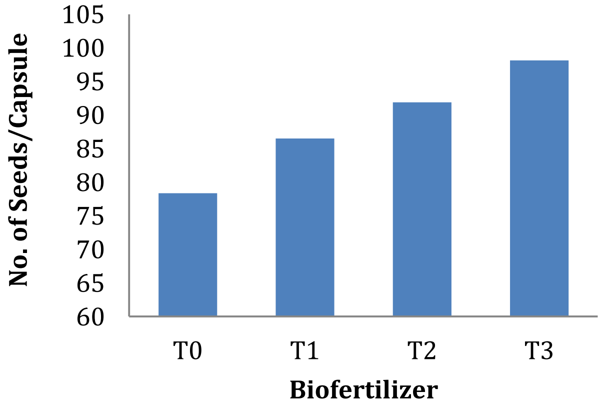

Figure 03. Effect of biofertilizer on number of seeds per capsule of black cumin (*Nigella sativa* L.). (LSD value= 0.9948)

Note: T_0 = 0 kg biofertilizer ha^{-1} (control), T_1 = 4 kg biofertilizer ha^{-1}, T_2 = 8 kg biofertilizer ha^{-1} and T_3 = 12 kg biofertilizer ha^{-1}

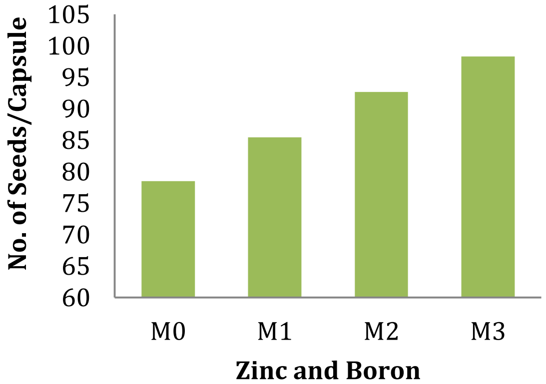

Figure 04. Effect of zinc and boron combination on number of seeds per capsule of black cumin (*Nigella sativa* L.). (LSD value=0.9948)

Note: M_0 = 0 kg ha^{-1} (control), M_1 = Zn_{4kg} ha^{-1}, M_2 = B_{2kg} ha^{-1} and M_3 = $Zn_{4kg}B_{2kg}$ ha^{-1}

Seeds per capsule

The maximum number of seeds capsule^{-1} (98.15) was recorded from T_3 (12 kg biofertilizer ha^{-1}) treatments and the T_0 (control) treatment gave the lowest one (78.35) (Figure 03). The results of this study showed that the yield and yield components significantly enhanced with the application of bio-fertilizers. Bulgarelli et al. (2017) reported that bio-fertilizers enhance nutrient availability which is an effective factor in stimulating plant growth and photosynthesis, improves the conditions for growth, and consequently increased yield components and seed yield of both species. These results are under Mehta et al. (2012) and Godara et al. (2018). The maximum number of seeds capsule^{-1} (98.29) was recorded in M_3 ($Zn_{4kg}B_{2kg}$ ha^{-1}) treatment. When the lowest number of seeds capsule^{-1} (78.50) was recorded in M_0 (control) (Figure 04). The maximum seed capsule^{-1} (109.83) obtained from T_3M_3 (12 kg biofertilizer ha^{-1} and $Zn_{4kg}B_{2kg}$ ha^{-1}) treatment combination. The minimum seed $capsule^{-1}$ (73.92) was obtained from T_0M_0 (control) treatment combination which was statistically identical (74.75) to T_0M_1 (control and Zn_{4kg}) treatment combination.

Weight of 1000 seed (g)

Application of different levels of bio-fertilizer significantly influenced 1000 seed weight of black cumin (Table 08). The maximum seed weight (3.18 g) was recorded from the T_3 (12 kg bio-fertilizer ha-1) treatment, whereas the T_0 (control) treatment gave the minimum weight of 1000 seeds (2.31 g). Applying bio-fertilizer increases the photosynthetic potential and the resulting filling of the plant reproductive seeds, which improves 1000-seed weight (Moradzadeh et al., 2021). 1000 seed weight of black cumin was significantly influenced by different levels of zinc and boron (Table 08). The maximum weight of 1000 seeds (3.19 g) was recorded from M3 (Zn4kgB2kg ha-1) treatment, whereas the lowest 1000 seed weight (2.37 g) was recorded from M_0 (control) treatment. Naga et al. (2013) indicated that the effect of boron and zinc was significant on 1000-seed weight, seed yield per plant, and seed yield per hectare. The highest 1000 seed weight (3.55 g) was obtained from T_3M_3 (12 kg bio-fertilizer ha^{-1} and $Zn_{4kg}B_{2kg}$ ha^{-1}) treatment combination, which was statistically similar to (3.63 g) to T_3M_2 (12 kg bio-fertilizer ha^{-1} and Zn_{4kg} ha^{-1}) treatment combination. The lowest 1000 seed weight (2.12 g) was recorded from T_0M_0 (control) treatment combination.

Table 08. Effect of bio-fertilizers, zinc and boron on weight of 1000 seeds, weight of seeds $plant^{-1}$ and weight of seeds $plot^{-1}$ of black cumin *(Nigella sativa L.)*

Treatment	Weight of 1000 seeds (g)	Weight of seeds $plant^{-1}$ (g)	Weight of seeds $plot^{-1}$ (g)
T_0	2.31 d	3.42 d	131.27 d
T_1	2.64 c	3.91 c	147.46 c
T_2	2.91 b	4.70 b	158.35 b
T_3	3.18 a	5.13 a	167.90 a
LSD (0.05)	0.0317	0.0356	0.5754
CV %	6.25	5.58	6.32
Treatment			
M_0	2.37 d	3.47 d	131.58 d
M_1	2.58 c	3.88 c	144.86 c
M_2	2.90 b	4.68 b	159.83 b
M_3	3.19 a	5.14a	168.69 a
LSD (0.05)	0.0317	0.0356	0.5754
CV %	6.25	5.58	6.32

Note: T_0 = 0 kg biofertilizer ha^{-1} (control), T_1 = 4 kg biofertilizer ha^{-1}, T_2 = 8 kg biofertilizer ha^{-1} and T_3 = 12 kg biofertilizer ha^{-1},. Note: M_0 = 0 kg ha^{-1} (control), M_1 = Zn_{4kg} ha^{-1}, M_2 = B_{2kg} ha^{-1} and M_3 = $Zn_{4kg}B_{2kg}$ ha^{-1} In a column means having similar letter (s) are statistically similar and those having dissimilar letter (s) differ significantly by LSD at 0.05 levels of probability

Weight of seeds $plant^{-1}$ (g)

Seed yield per plant was significantly influenced due to the application of different levels of biofertilizer (Table 08). Yield was increased with increasing plant nutrients. Results showed that the maximum seed yield per plant (5.13 g) was recorded from T_3 (12 kg bio-fertilizer ha^{-1}) treatment and the lowest seed yield per plant (3.42 g) was recorded from T_0 (control) treatment. When seeds are inoculated with biological fertilizers, it may increase the motility and the absorptive capability of the root system to improve nutrient absorption of phosphor, resulting in increased photosynthesis and seed yield per plant (Faravani et al., 2012). Seed yield $plant^{-1}$ was significantly influenced by different levels of zinc and boron (Table 08). It was observed that higher zinc and boron gave maximum yield. The maximum seed yield $plant^{-1}$ (5.14 g) was recorded from M_3 ($Zn_{4kg}B_{2kg}$ ha^{-1}) treatment whereas the lowest seed yield $plant^{-1}$ (3.47 g) was recorded from M_0 (control) treatment. The highest yield was obtained from applying zinc or boron (Shil et al., 2007). This might be because boron helped increase plants' reproductive development and finally increased the total yield (Jena et al., 2009). Naiknaware et al. (2015) reported that the application of boron increased the yield per plant. The highest yield $plant^{-1}$ (6.10 g) was obtained from T_3M_3 (12 kg biofertilizer ha^{-1} and $Zn_{4kg}B_{2kg}$ ha^{-1}) treatment combination. The lowest seed yield $plant^{-1}$ (3.04 g) was recorded from T_0M_0 (control) treatment combination (Table 09).

Table 09. Combined effect of bio-fertilizers with zinc and boron on weight of 1000 seeds, number of seeds capsule^{-1}, weight of seeds plant^{-1}, weight of seeds plot^{-1} of black cumin (*Nigella sativa* L.)

Treatments	Weight of 1000 seeds (g)	Number of seeds capsule^{-1}	Weight of seeds plant^{-1} (g)	Weight of seeds plot^{-1} (g)
T_0M_0	2.12 l	73.92 l	3.04 k	121.32 m
T_0M_1	2.19 k	74.75 l	3.15 j	124.86 l
T_0M_2	2.31 ij	79.33 j	3.54 i	135.12 j
T_0M_3	2.62 g	85.42 gh	3.98 g	143.76 h
T_1M_0	2.27 j	76.83 k	3.13 j	129.00 k
T_1M_1	2.42 h	84.92 h	3.83 h	144.20 h
T_1M_2	2.73 f	88.67 f	4.11 f	151.99 g
T_1M_3	3.14 c	95.58 de	4.57 d	164.63 e
T_2M_0	2.37 hi	80.50 j	3.60 i	134.03 j
T_2M_1	2.76 f	87.33 fg	4.16 f	151.11 g
T_2M_2	3.05 d	97.33 d	5.14 c	169.78 d
T_2M_3	3.47 b	102.33 c	5.89 b	178.49 c
T_3M_0	2.72 f	82.75 i	4.09 f	141.99 i
T_3M_1	2.93 e	94.83 e	4.37 e	159.29 f
T_3M_2	3.53 ab	105.17 b	5.93 b	182.43 b
T_3M_3	3.55 a	109.83 a	6.10 a	187.89 a
LSD (0.05)	0.0633	1.9895	0.0713	1.1508
CV %	6.25	4.02	5.58	6.32

Note: T_0 = 0 kg biofertilizer ha^{-1} (control), T_1 = 4 kg biofertilizers ha^{-1}, T_2 = 8 kg biofertilizer ha^{-1} and T_3 = 12 kg biofertilizers ha^{-11}, M_0 = 0 kg ha^{-1} (control), M_1 = Zn_{4kg} ha^{-1}, M_2 = B_{2kg} ha^{-1} and M_3 = $Zn_{4kg}B_{2kg}$ ha^{-1} In a column means having similar letter (s) are statistically similar and those having dissimilar letter (s) differ significantly by LSD at 0.05 levels of probability.

Weight of seeds plot^{-1} (g)

The maximum seed yield per plot (167.90 g) was recorded from T_3 (12 kg biofertilizer ha^{-1}) treatment and the lowest seed yield per plot (131.27 g) was recorded from T_0 (control) treatment. Bio-fertilizers have more advantages for plant feeding, such as improving the root system and creating deeper and more abundant roots. Phosphorus-solubilizing bacteria increase the available nitrogen and phosphorus in the soil, which could enhance crop production (Faravani et al., 2012; Bulgarelli et al., 2017). Seed yield plot^{-1} was significantly influenced by different levels of zinc and boron (Table 08). It was observed that higher zinc and boron gave maximum yield. The maximum seed yield plot^{-1} (168.69 g) was recorded from M_3 ($Zn_{4kg}B_{2kg}$ ha^{-1}) treatment, whereas the lowest seed yield plot^{-1} (131.58 g) was recorded from M_0 (control) treatment. This might be due to the application of zinc and boron along with the recommended dose of NPK, which enhanced the yield attributing characteristics. The highest yield plot^{-1} (187.89 g) was obtained from T_3M_3 (12 kg biofertilizer ha^{-1} and $Zn_{4kg}B_{2kg}$ ha^{-1}) treatment combination. The lowest seed yield plot^{-1} (121.32 g) was recorded from T_0M_0 (control) treatment combination (Table 09).

Cost benefit analysis

Combining biofertilizer with zinc and boron maximum gross return (Tk. 219205) was obtained in T_3M_3 (12 kg biofertilizer ha^{-1} and $Zn_{4kg}B_{2kg}$ ha^{-1}). The lowest gross return (Tk. 115770) was obtained in the combination of T_0M_0 (control) treatment (Table 10). Different treatment combinations give different types of net returns with the help of equation number (1). Combining biofertilizer with zinc and boron, the highest net return (Tk. 136719) was obtained from the T_3M_3 (12 kg biofertilizer ha^{-1} and $Zn_{4kg}B_{2kg}$ ha^{-1}) treatment combination. The lowest net return (Tk. 49805) was obtained in the T_0M_0 (control with Zn_{4kg} ha^{-1}) treatment combination (Table 10). Combining biofertilizer with zinc and boron, the highest benefit cost ratio (2.66) was attained from the T_3M_3 (12 kg biofertilizer ha^{-1} and $Zn_{4kg}B_{2kg}$ ha^{-1}) treatment combination. The lowest benefit cost ratio (1.72) was obtained from T_0M_0 (control) treatment combination (Table 10).

Table 10. Cost and return analysis of black cumin considering bio-fertilizers with zinc and boron

Treatments	Cost of production (Tk)	Yield (t ha^{-1})	Gross income (Tk)	Net income (Tk)	BCR
T_0M_0	65965	771.8	115770	49805	1.76
T_0M_1	68665	832.4	124860	56195	1.82
T_0M_2	67909	900.8	135120	67211	1.99
T_0M_3	70608	958.40	143760	73152	2.04
T_1M_0	69744	860	129000	59256	1.85
T_1M_1	72444	961.3	144195	71751	1.99
T_1M_2	71688	1013.3	151995	80307	2.12
T_1M_3	74927	1097.5	164625	89698	2.20
T_2M_0	71904	893.5	134025	62121	1.86
T_2M_1	74603	1007.4	151110	76507	2.03
T_2M_2	73848	1131.9	169785	95937	2.30
T_2M_3	74927	1189.9	178485	103558	2.38
T_3M_0	76223	946.6	141990	65767	1.86
T_3M_1	78923	1061.9	159285	80362	2.02
T_3M_2	79247	1216.2	182430	103183	2.30
T_3M_3	82486	1252.6	219205	136719	2.66

Note: T_0 = 0 kg biofertilizer ha^{-1} (control), T_1 = 4 kg biofertilizer ha^{-1}, T_2 = 8 kg biofertilizer ha^{-1} and T_3 = 12 kg biofertilizer ha^{-1}, M_0 = 0_{kg} ha^{-1} (control), M_1 = Zn_{4kg} ha^{-1}, M_2 = B_{2kg} ha^{-1} and M_3 = $Zn_{4kg}B_{2kg}$ ha^{-1}

IV. Conclusion

Considering the above results of this experiment, T_3 (12 kg bio-fertilizers ha^{-1}) treatment was more effective than without bio-fertilizers T_0 (control), whereas the zinc and boron M_3 ($Zn_{4kg}B_{2kg}$ ha^{-1}) gave higher seed yield per hectare than without nutrient M_0 (control). So, the best treatment combination was obtained from T_3M_3 (12 kg bio-fertilizers ha^{-1} with $Zn_{4kg}B_{2kg}$ ha^{-1}), having a yield per plot of 168.69 (g). Considering the results, it could be made a recommendation that further study may be needed to ensure the different micronutrient combinations and bio-fertilizer concerning growth and seed yield and quality performance of black cumin in different agro-ecological zones (AEZ) of Bangladesh for regional adaptability.

Acknowledgement

The experiment was conducted as thesis work for the partial fulfillment of master degree by the first author Niloy Banik. The authors are highly grateful to the Department of Horticulture, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh. This experiment was supported and funded by the Ministry of Science and Technology under the NST fellowship.

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